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Structure activity studies of the serine-AIB dipeptide domain in 2,3-dihydroisothiazole based growth hormone secretagogues

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Abstract—A series of growth hormone secretagogues (GHSs) based on 2,3-dihydroisothiazole has been synthesized in the search for a potential treatment of growth hormone deficiency or frailty in the elderly. This paper describes the evaluation of the SAR of the benzyl-D-Ser-aminoisobutyric acid dipeptide fragment. Introduction of substituents in the peptide backbone and in the phenyl ring has been investigated, as well as replacements for the benzyl group and for the AIB residue. A number of modifications resulted in enhanced potency over the parent benzyl-D-Ser-AIB derivative.

1. Introduction

Growth hormone secretagogues (GHSs) have been widely studied during the past several years as an alternative to (recombinant) growth hormone for the treatment of growth hormone deficiency and frailty in the elderly. These compounds release endogenous growth hormone through interaction with the GHS receptor (GHS-R1a), a G-protein-coupled receptor, occurring predominantly in the pituitary gland and in the hypothalamus. The first GHSs discovered were oligopeptides, called growth hormone releasing peptides (GHRPs), like the hexapeptide GHRP-6.[1] Later, small orally bioavailable peptidomimetic GHSs were also identified.[2] Interestingly, it was only in 1999 that the endogenous GHS-receptor ligand, ghrelin, was discovered.[3] This 28-amino acid peptide contains a unique octanoyl-serine residue and is secreted in the stomach.

Many of the small molecule GHSs described in the literature, some of which have been advanced to clinical investigations,[4] contain as a common feature a dipeptide formed by *O*-benzyl-D-serine and aminoisobutyric acid (AIB), or carbon-analogues such as those found in LY 444711.[5,6] Examples include MK-0677[7] and CP-424391[8] shown in Figure 1. The structure-function studies of ghrelin have been summarized[9] and suggest that the dipeptide corresponds to the N-terminus of the GHRPs and ghrelin. Most research groups have made minimal changes to this structural motif, while focussing their attention on modifications of other part of the molecule.

In this paper, we present a part of the results of our work on a series of 2,3-dihydroisothiazole GHSs.[10–12] Based on compound **1** that has shown potent growth hormone releasing activity in a rat pituitary cell assay, we have explored both the steric and electronic requirements of the dipeptide moiety in this series. One approach was to systematically introduce methyl groups into different positions of the serine. In addition, we varied the chain length of the linker, introduced substituents into the benzyloxy group and replaced it by heterocycles.

2. Chemistry

To study the effect of substituted benzyl serine or of heterocyclic benzyl replacements, a series of D-serine analogues was prepared. The building blocks were

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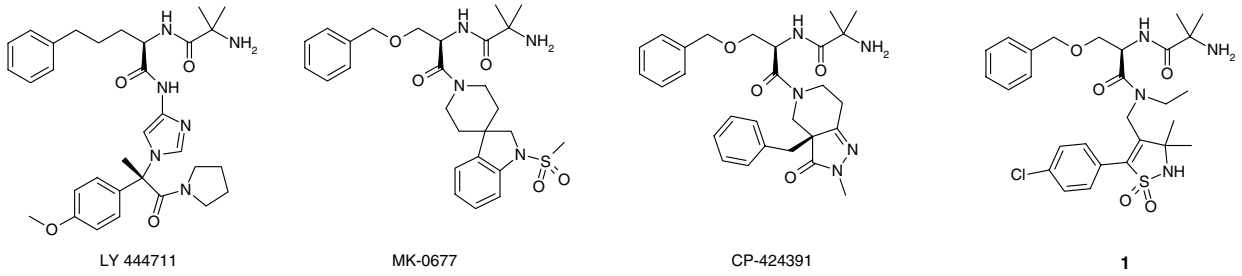


Figure 1. Growth hormone secretagogues with a common dipeptide structural motif.

obtained by alkylation of *N*-Boc-D-serine (**2**) with substituted benzyl or heteroarylmethyl halides (Scheme 1). Subsequent coupling and deprotection steps with the amine **4**[10] and with Boc-AIB (**7**) yielded the products **9a**–**z** and **11a**–**j**.

N-Boc-D-2-amino-5-phenyl-pentanoic acid (**15a**) and its fluorinated analogues **15b**–**d** were prepared as shown in Scheme 2 analogous to known procedures. Diethyl acetamidomalonate (**12**) was alkylated with (substituted) 1-bromo-3-phenylpropanes, and the products were subsequently decarboxylated under basic conditions. Acylase-mediated deacylation[13] of the racemic mixture afforded the D-*N*-acetyl amino acids **14a**–**d** selectively. They were deacylated, the amino groups were Boc-protected, and the resulting esters were cleaved to afford **15a**–**d**, which were then coupled with amine **4** under standard conditions. Deprotection and subsequent coupling to Boc-AIB according to the methods in Scheme 1, followed by final Boc removal, provided the products **16a**–**d**.

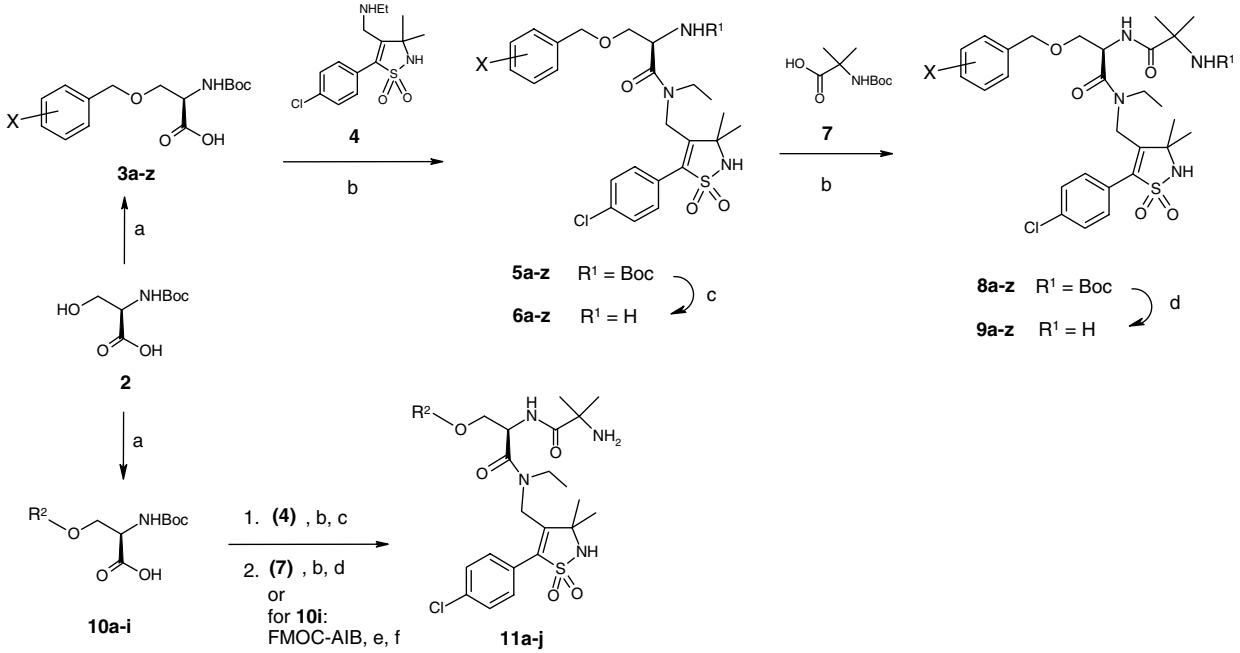
In a route analogous to that used to access the D-serine series, *N*-Boc-benzyl-D-allo-threonine (**17**) was converted to derivative **18**, as depicted in Scheme 3.

To study the effect of *N*-methylserine, the alanine analogues **22** and **23** were prepared following the route outlined in Scheme 3. Boc-protected benzyl-D-serine (**19**) was converted into the oxazolidinone **20** by refluxing with paraformaldehyde and *p*-TosOH. Subsequent reductive cleavage with Et_3SiH and TFA,[14] followed by reprotection with Boc, led to the *N*-Me-serine intermediate **21** in good yield.

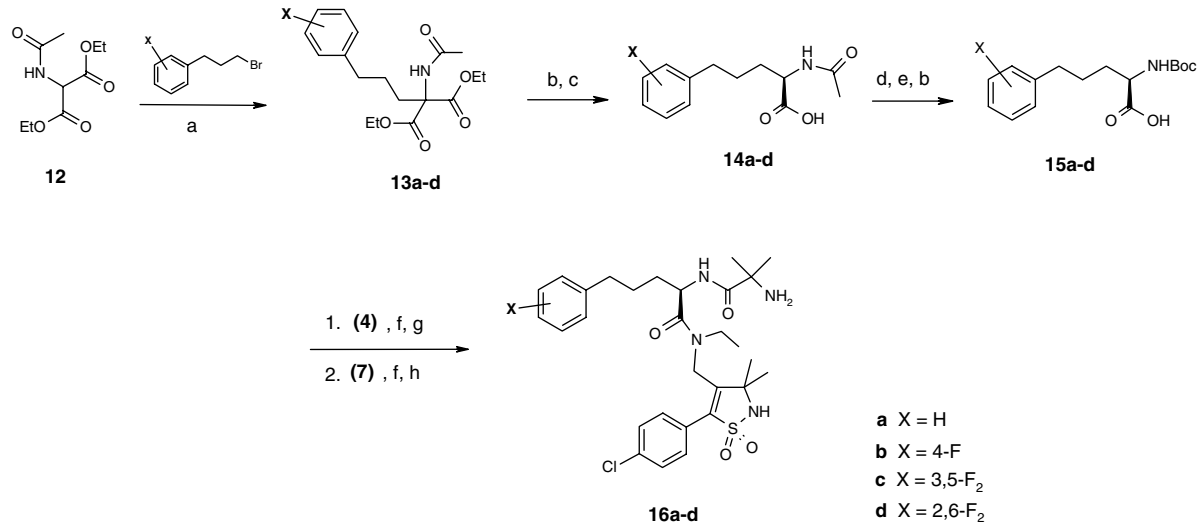
Intermediate **6** and its 2,6-difluorobenzyl analogue **6s** were applied in coupling reactions with a number of Boc-protected amino acids to afford compounds with replacements for the aminoisobutyric acid such as 2-fluoromethyl-3-fluoro-alanine[15] (**24**), 1-amino-cyclopropane-carboxylic acid (**25**), α-methyl-serine (**26** and **27**) and the alanine derivatives **28** and **29**.

3. Results and discussion

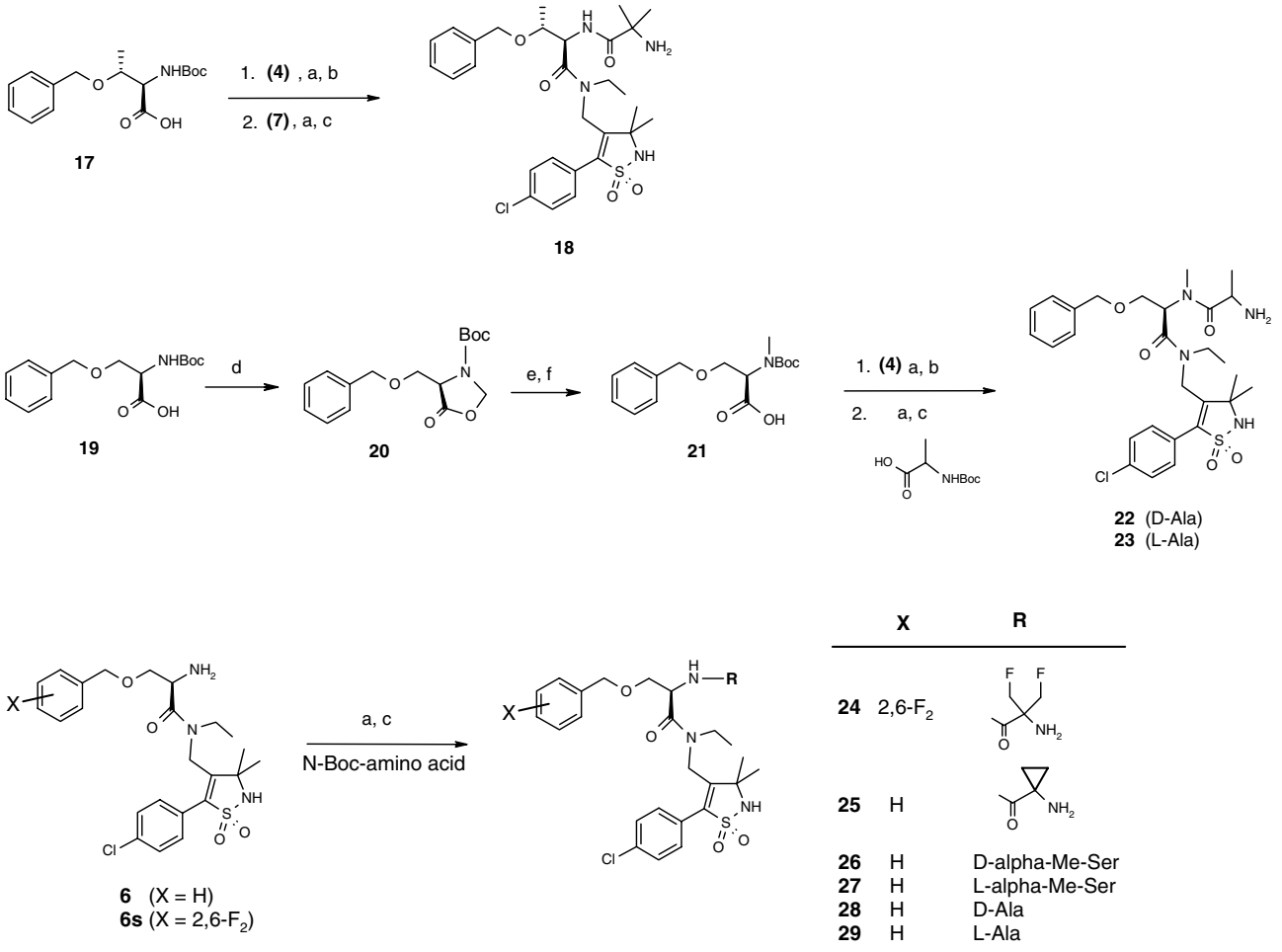
The N-terminal structure of ghrelin, the natural ligand for the GHS receptor, begins with a Gly-Ser-Ser tripeptide, in which the serine in the third position is esterified with octanoic acid. Simple overlay of the structures of



Scheme 1. Synthesis of D-serine derivatives. Reagents and conditions: (a) NaH, DMF, rt, overnight; (b) DCC, HOBt, CH_2Cl_2, rt; (c) TFA, CH_2Cl_2, rt; (d) HCl, EtOH, rt; (e) TBTU, NEt_3, rt; (f) DMF, $EtNH_2$, rt.



Scheme 2. Synthesis of D-2-amino-5-phenyl-pentanoic acid analogues. Reagents and conditions: (a) Na, EtOH, reflux; (b) NaOH, reflux; (c) acylase, $CoCl_2$, KOH; (d) HCl, EtOH, reflux; (e) $(Boc)_2O$, NEt_3, THF, rt; (f) DCC, HOBt, CH_2Cl_2, rt; (g) TFA, CH_2Cl_2, rt; (h) HCl, EtOH, rt.



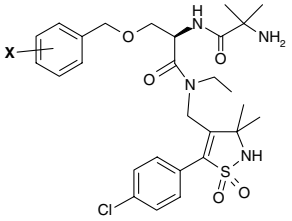
Scheme 3. Synthesis of methyl analogues of **1** and AIB replacements. Reagents and conditions: (a) DCC, HOBt, CH_2Cl_2, rt; (b) TFA, CH_2Cl_2, rt; (c) HCl, EtOH, rt; (d) paraformaldehyde, *p*-TosOH, toluene, reflux; (e) Et_3SiH, TFA, CH_2Cl_2; (f) $(Boc)_2O$, NEt_3, THF, rt.

benzylserine derived GHSs with that of ghrelin suggests that the benzylserine moiety of the GHSs binds in the same region of the receptor as the octanoyl chain. This has also been demonstrated by an octanoyl-serine derivative of MK-0677 that retained some activity in the functional assay.[16] We explored the steric and electronic

requirements of binding to this pocket in a series of benzyl-serine-2,3-dihydro-isothiazole-based GHSs, which contained primarily hydrophobic residues in the dipeptide. All compounds were tested for their ability to promote secretion of growth hormone on isolated rat pituitary cells.

We first studied the influence of substituents on the phenyl ring of the benzyl serine moiety (Table 1). We found that substitution in the *para*-position reduced the level of in vitro activity, relative to substitution in the *meta*- or *ortho*-position (compare **9a** with **9b** or **9c**; **9i** with **9j**; **9e** with **9k**). Generally, polar substituents, such as trifluoromethoxy (**9f**), carboxamide (**9m**), or the 4-cyano derivative **9i**, led to decreased levels of in vitro activity. An exception is the 2-cyano analogue **9j** (EC_{50} = 1.9 nM). We also prepared various *ortho*-substituted benzyl derivatives. Introduction of substituents in only one of the *ortho*-positions caused little or no increase in in vitro activity compared with the parent compound **1**. However, when a substituent like halogen was incorporated into both *ortho*-positions, the resulting compounds showed a significant increase in the level of in vitro activity when compared with compound **1**. In fact, the 2,6-dihalogenated analogues **9t** and **9w**–**9y** demonstrated activity in the subnanomolar range. We assume that this effect can be explained by stabilisation of a favourable conformation of the aromatic residue through hindered rotation of the benzylic C–C bond. Therefore, the observed gain in in vitro activity is more pronounced with chlorine substituents compared to the smaller fluorine atoms.

Table 1. Activities of substituted benzyl derivatives in the pituitary cell assay



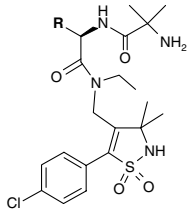
Compound	X	EC_{50} (nM)	SEM
1	H	3.5	0.7
9a	4-F	12.1	5.4
9b	3-F	3.1	0.9
9c	2-F	3.1	1.5
9d	4-Cl	8.9	1.2
9e	4-CF_3	38	18[a]
9f	4-OCF_3	>83	
9g	4-Cl-2-F	8.2	3.5
9h	2-Cl-4-F	8.1	2.5
9i	4-CN	>71	
9j	2-CN	1.9	0.5
9k	2-CF_3	8.3	2.4
9l	2-CH_3	8.8	3.2
9m	2-$CONH_2$	55	24[a]
9n	2,3-Cl_2	2.0	0.7
9o	2,3-F_2	2.7	0.4
9p	2,4-F_2	8.0	2.4
9q	2,5-F_2	2.0	0.8
9r	3,5-F_2	7.0	1.1
9s	2,6-F_2	1.1	0.5
9t	2,6-Cl_2	0.1	0.05[a]
9u	2,4,5-F_3	18	6.8
9v	2,3,5-F_3	1.3	0.3
9w	2,3,6-F_3	0.3	0.07
9x	2-Cl-3,6-F_2	0.5	0.5
9y	2-Cl-6-F	<0.1	
9z	2,6-F_2-3-CH_3	1.4	0.4

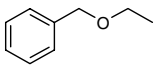
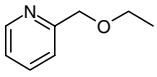
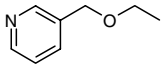
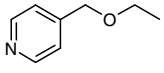
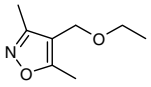
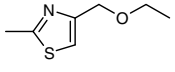
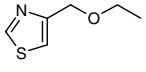
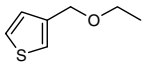
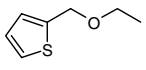
[a] n = 2.

The phenyl ring of the *O*-benzyl-D-serine moiety was replaced with a series of heterocyclic groups. The results are shown in Table 2. Whereas all the nitrogen-containing heterocycles tested showed moderate in vitro activity (EC_{50} = 22 to >100 nM), the hydrophobic 3- and 2-thienyl derivatives **11g** and **11h** proved to have comparable in vitro activity (EC_{50} values 3.4 and 3.6 nM, respectively) to the parent compound **1**.

Replacement of the ether linker in the benzylserine with its carbon analogue, 2-amino-5-phenyl-pentanoic acid,

Table 2. Heterocyclic replacements for the benzyl group



Compound	R	EC_{50} (nM)	SEM
1		3.5	0.7
11a		38	28[a]
11b		43	3[a]
11c		54	22
11d		>100	
11e		22	8
11f		47	17
11g		3.4	0.4
11h		3.6	1.5

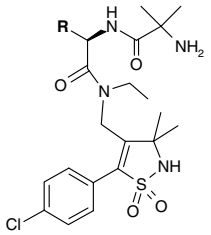
[a] n = 2.

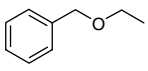
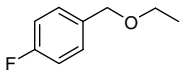
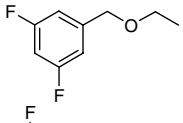
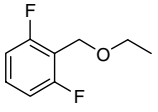
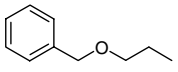
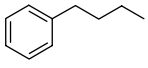
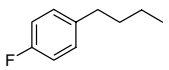
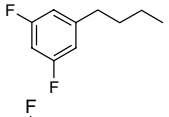
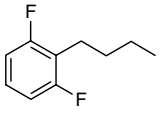
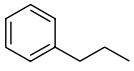
resulted in a modest improvement of in vitro activity (Table 3, entries **1** and **16a**). This trend of increased activity in the carbon series was also shown with a number of fluorinated carbon analogues. Indeed, in all cases the carbon analogues showed at least 2-fold more in vitro activity than their ether counterparts (Table 3). The greatest increase in in vitro activity, approximately 10-fold, was seen in the case of 2,6-difluoro substitution (compare compounds **9s** and **16d**). Compound **16d** stimulated GH secretion in the pituitary cell with an EC_{50} of 0.1 nM.

In addition, we studied the effect of the chain length of the linker on the activity and found that the three-atom linker between phenyl and asymmetric carbon was already optimal. Extension of the chain by one atom via incorporation of benzyl-D-homoserine (**30**), as well as shortening the linker by one methylene group as in the D-homophenylalanine derivative (**31**), resulted in a reduction in the level of in vitro activity (Table 3).

Finally, we investigated how additional substituents were tolerated along the dipeptide chain, using the methyl group as an example (Table 4). We found that an

Table 3. Linker variations

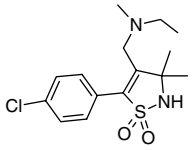


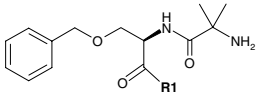
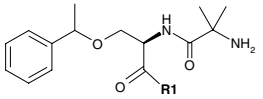
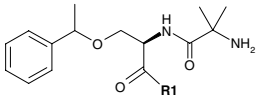
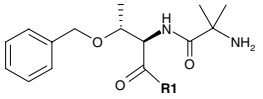
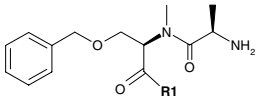
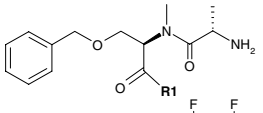
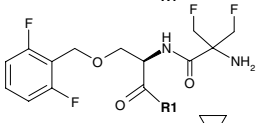
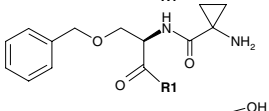
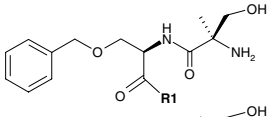
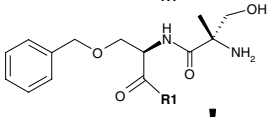
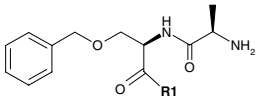
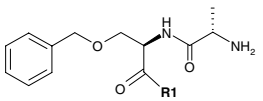
Compound	R	EC_{50} (nM)	SEM
1		3.5	0.73
9a		12.1	5.4
9r		7.0	1.1
9s		1.1	0.5
30		>56	
16a		1.1	0.5
16b		2.3	0.7
16c		3.7	1.3
16d		0.1	0.09
31		58	38[a]

[a] $n = 2$.

Table 4. Methyl derivatives of **1** and AIB variations

R1 =



Cpd.		EC_{50} (nM)	SEM
1		3.5	0.73
11i		0.9	0.12
11j		0.3	0.19
18		>100	
22		>96	
23		3.4	2.5
24		78	13[a]
25		>100	
26		0.7	0.1
27		20.3	2.6
28		>100	
29		4.4	1.2

[a] $n = 2$.

additional methyl group in the benzylserine was only tolerated if it were distal to the α-carbon. Compared to the unsubstituted compound **1**, the two isomers **11i** and **11j** with the methyl group in the benzylic position had significantly improved in vitro activity with EC_{50} values of 0.9 and 0.3 nM, respectively. In contrast, for the D-allo-threonine derivative **18** a reduction of in vitro activity was observed.

To study the effect of N-methylation, we prepared the *N*-Me-Ala analogues **22** and **23**. Comparison with their NH-Ala analogues **28** and **29** shows that L-alanine (**23** and **29**) provides comparable in vitro activity with parent **1**, whereas the D-alanine analogues **22** and **28** reduced the level of activity in the pituitary cell assay. N-Methylation in the serine is well-tolerated for good AIB replacements.

The sensitivity of the GHS activity to minor modifications within the AIB moiety was illustrated by compounds **24** and **25**, in which fluorination of the methyl groups or their replacement by cyclopropane, respectively, resulted in a reduction in the level of in vitro activity, including an example with the 2,6-difluorophenyl ring as in compound **24**. Interestingly, however, selective hydroxylation of one of the AIB-methyl groups results in a 5-fold increase in activity versus parent **1** (*R*-isomer **26**), whereas hydroxylation of the other methyl group results in a 6-fold reduction (*S*-isomer **27**). The preference of the *R*-isomer is in agreement with results from other series.[17]

4. Conclusions

The SAR of the benzyl-D-Ser-AIB dipeptide fragment in a series of dihydroisothiazole-based GHSs has been evaluated thoroughly. Starting from GHS **1** with an EC_{50} of 3.5 nM, a number of modifications led to additional improvement of the in vitro activity. In agreement with the assumption that the benzylserine moiety occupies the same region at the GHS receptor as the octanoyl side chain of the N-terminus of ghrelin, nonpolar replacements for the benzyl residue were well-tolerated. Whereas heterocycles, such as, pyridines and thiazoles, decreased the in vitro activity, the thienyl analogues **11g** and **11h** were found to have comparable in vitro activity to **1**, and also the α-methylbenzyl analogues **11i** and **11j** are good examples of how hydrophobic groups can contribute to efficient ligand binding at the receptor. The two isomers **11i** and **11j** show a 3-fold difference in activity. The absolute stereochemistry and the influence of larger groups in that position remain to be evaluated.

The greatest gain in in vitro activity was achieved through the introduction of 2,6-dihalogenated benzyl groups as in **9t**, **9w** and **9y**. This gain cannot be attributed to electronic effects only, because other derivatives with electron-deficient phenyl rings, such as, **9g**, **9h** and **9p**, do not display the same boost in activity. Instead, steric effects seem to play a role, which is demonstrated by a reduction in the level of in vitro activity with the introduction of *para*-substituents. We assume that through hindered rotation of the benzylic C–C bond in case of the 2,6-disubstituted analogues a more favourable orientation of the aromatic residue is stabilised. This becomes especially apparent for the chlorine derivatives, whereas the conformation of **9s** with the smaller fluorine atoms can be considered as being more similar to the unsubstituted **1**. Such an effect could also explain the improved activity of the α-methylbenzyl analogues **11i** and **11j**.

We demonstrated that the chain length of the linker in benzylserine was optimal, and that seemingly small changes in the AIB moiety decreased activity, with the exception of the hydroxylated compound **26**, which displayed a 5-fold improvement in EC_{50} over the corresponding AIB analogue **1**.

A detailed summary of our results describing the SAR of the dihydroisothiazole moiety will be published elsewhere.

5. Experimental

5.1. General chemistry methods

All reagents and solvents were obtained from commercial sources and were used without further purification. ^{1}H NMR spectra were recorded on a Bruker Avance 300 MHz or on a Varian Inova 500 MHz in $CDCl_3$ or DMSO-d_6 as solvent using the deuterium lock signal as internal standard. Chemical shift data are given as δ (ppm) values. Low-resolution mass spectra were obtained a Perkin-Elmer Sciex API 150 MCA using electrospray ionisation (ESI). Preparative chromatographic separations were performed using flash chromatography with silica gel (Millipore, 60A, 35–70 μm).

5.2. Method A: serine alkylation

N-Boc-D-serine (**2**) (1 g, 4.9 mmol) was dissolved in DMF and cooled to 5 °C, NaH (60% in mineral oil, 0.49 g, 12.25 mmol) was added and the mixture was stirred for 30 min. The substituted benzyl halide or the corresponding heteroarylmethyl bromide (1.05 equiv) was added and the mixture was stirred overnight at rt. After evaporation, the residue was dissolved in water (50 ml), extracted with *t*-butylmethylether, acidified with 10% citric acid and extracted with ethyl acetate. The ethyl acetate layer was dried (Na_2SO_4) and evaporated. Chromatography on silica with a CH_2Cl_2–EtOH gradient yielded the product.

5.3. Method B: coupling with amine 4 and deprotection

5.3.1. 2-(*R*)-2-Amino-3-benzyloxy-*N*-[5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydro-isothiazol-4-ylmethyl]-*N*-ethyl-propionamide (6, X = H). [5-(4-Chloro phenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydro-isothiazol-4- ylmethyl]-ethylamine[10] (**4**, 500 mg, 1.59 mmol) was suspended in isopropylacetate (10 ml). Water (5 ml), DCC (361 mg, 1.75 mmol), HOBT (237 mg, 1.75 mmol) and *N*-Boc-*O*-benzyl-D-serine (517 mg, 1.75 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was filtered, rinsed with isopropy-

lacetate, and the aqueous phase was separated. The organic layer was washed with citric acid (0.1 M) and satd $NaHCO_3$, dried over Na_2SO_4 and concentrated. The residue was dissolved in CH_2Cl_2 (10 ml) and TFA (7.5 ml) was added at 0 °C. The solution was allowed to warm to room temperature and stirred overnight. After evaporation, the residue was dissolved in CH_2Cl_2, washed with satd $NaHCO_3$ and satd NaCl solutions, dried over Na_2SO_4 and concentrated to yield **6** (440 mg, 56%) as an amorphous solid. ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H), 7.43 (m, 2H), 7.38–7.24 (m, 5H), 4.41 (m, 3H), 4.19 (d, 1H, J = 5.8 Hz), 3.55 (m, 1H), 3.23 (m, 2H), 3.04 (m, 2H), 1.40 (m, 6H), 0.82 (t, 3H). MS m/z 492 (M^++Na).

5.4. Method C: coupling with Boc-AIB and deprotection

5.4.1. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-phenylmethoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (1). 2-(*R*)-2-Amino-3-benzyloxy-*N*-[5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydro-isothiazol-4-ylmethyl]-*N*-ethyl-propionamide (**6**, 440 mg, 0.89 mmol) was suspended in isopropylacetate (6 ml). Water (6 ml), DCC (202 mg, 0.98 mmol), HOBT (132 mg, 0.98 mmol) and *N*-Boc-α-amino-isobutyric acid (**7**, 200 mg, 0.98 mmol) were added and the mixture was stirred overnight at room temperature. The mixture was filtered, rinsed with isopropylacetate, and the aqueous phase was separated. The organic layer was washed with citric acid (0.1 M) and satd $NaHCO_3$, dried over Na_2SO_4 and concentrated. The residue was purified by chromatography on silica gel (CH_2Cl_2/EtOH 98:2). The product was dissolved in 10% HCl in EtOH (4 ml) and stirred overnight at room temperature. The mixture was poured into diethyl ether (400 ml), stirred for 1 h and the precipitate was filtered off and dried at 50 °C under vacuum to yield **1** as a white solid (320 mg, 59%). ^{1}H NMR (DMSO-d_6) δ 7.51 (d, 2H, J = 8.5 Hz), 7.43 (d, 2H, J = 8.5 Hz), 7.34–7.24 (m, 5H), 4.66 (m, 1H), 4.53 (d, 1H, J = 16.2 Hz), 4.41 (s, 2H), 4.05 (d, 1H, J = 16.2 Hz), 3.34 (m, 1H), 3.05 (m, 3H), 1.45 (s, 3H), 1.43 (s, 3H), 1.39 (s, 6H), 0.92 (t, 3H, J = 6.9 Hz). MS m/z 577 (MH^+).

5.4.2. *N*-*tert*-Butoxycarbonylamino-*O*-(4-fluorobenzyl)-D-serine (3a). The title compound was prepared from **2** and 4-fluorobenzyl bromide by Method A (yield = 42%). ^{1}H NMR ($CDCl_3$) δ 8.85 (br s, 1H), 7.29 (m, 2H), 7.32 (dd, 2H, J^1 = 8.5 Hz, J^2 = 8.7 Hz), 5.44 (d, 1H, J = 8.3 Hz, NH), 4.49 (s, 2H), 4.47 (m, 1H), 3.92 (m, 1H), 3.71 (m, 1H), 1.45 (s, 9H). MS m/z 312 (M^-−1).

5.4.3. *N*-*tert*-Butoxycarbonylamino-*O*-(3-fluorobenzyl)-D-serine (3b). The title compound was prepared from **2** and 3-fluorobenzyl bromide by Method A (yield = 58%). ^{1}H NMR ($CDCl_3$) δ 8.89 (br s, 1H), 7.27 (m, 1H), 7.08–6.91 (m, 3H), 5.51 (d, 1H, 7.3 Hz), 4.51 (m, 3H), 3.93 (m, 1H), 3.73 (m, 1H), 1.45 (s, 9H). MS m/z 312 (M^-−1).

5.4.4. *N*-*tert*-Butoxycarbonylamino-*O*-(2-fluorobenzyl)-D-serine (3c). The title compound was prepared from **2** and 2-fluorobenzyl bromide by Method A (yield = 39%). ^{1}H NMR ($CDCl_3$) δ 8.84 (br s, 1H), 7.40–6.68 (m, 4H), 5.42 (d, 1H, J = 8.3 Hz), 4.61 (s, 2H), 4.48 (m, 1H), 3.96 (m, 1H), 3.74 (m, 1H), 1.45 (s, 9H). MS m/z 312 (M^-−1).

5.4.5. *N*-*tert*-Butoxycarbonylamino-*O*-(4-chlorobenzyl)-D-serine (3d). The title compound was prepared from **2** and 4-chlorobenzyl bromide by Method A (yield = 43%). ^{1}H NMR ($CDCl_3$) δ 9.61 (br s, 1H), 7.29 (m, 2H), 7.22 (m, 2H), 5.41 (d, 1H, J = 8.3 Hz), 4.48 (m, 3H), 3.90 (m, 1H), 3.71 (dd, 1H, J^1 = 3.4 Hz J^2 = 9.4 Hz), 1.45 (s, 9H). MS m/z 328 (M^-−1).

5.4.6. *N*-*tert*-Butoxycarbonylamino-*O*-(4-trifluoromethylbenzyl)-D-serine (3e). The title compound was prepared from **2** and 4-trifluoromethylbenzyl bromide by Method A (yield = 66%). ^{1}H NMR (DMSO-d_6) δ 12.6 (br s, 1H), 7.70 (m, 2H), 7.54 (m, 2H), 7.02 (d, 1H, J = 8.3 Hz), 4.59 (m, 2H), 4.21 (m, 1H), 3.70 (m, 2H), 1.37 (s, 9H). MS m/z 386 (M^++Na).

5.4.7. *N*-*tert*-Butoxycarbonylamino-*O*-(4-trifluoromethoxybenzyl)-D-serine (3f). The title compound was prepared from **2** and 4-trifluoromethoxybenzyl bromide by Method A (yield = 54%). ^{1}H NMR (DMSO-d_6) δ 12.7 (br s, 1H), 7.44 (m, 2H), 7.32 (m, 2H), 7.00 (d, 1H, J = 8.3 Hz), 4.45 (m, 2H), 4.19 (m, 1H), 3.68 (m, 2H), 1.37 (s, 9H). MS m/z 402 (M^++Na).

5.4.8. *N*-*tert*-Butoxycarbonylamino-*O*-(4-chloro-2-fluorobenzyl)-D-serine (3g). The title compound was prepared from **2** and 4-chloro-2-fluorobenzyl bromide by Method A (yield = 90%). ^{1}H NMR (DMSO-d_6) δ 12.8 (br s, 1H), 7.58–7.40 (m, 2H), 7.23 (m, 1H), 6.98 (d, 1H, J = 8.3 Hz), 4.51 (m, 2H), 4.19 (m, 1H), 3.74 (m, 2H), 1.37 (s, 9H). MS m/z 370 (M^++Na).

5.4.9. *N*-*tert*-Butoxycarbonylamino-*O*-(2-chloro-4-fluorobenzyl)-D-serine (3h). The title compound was prepared from **2** and 2-chloro-4-fluorobenzyl bromide by Method A (yield = 39%). ^{1}H NMR (DMSO-d_6) δ 12.8 (br s, 1H), 7.58–7.40 (m, 2H), 7.22 (m, 1H), 6.98 (d, 1H, J = 8.3 Hz), 4.53 (m, 2H), 4.21 (m, 1H), 3.74 (m, 2H), 1.37 (s, 9H). MS m/z 370 (M^++Na).

5.4.10. *N*-*tert*-Butoxycarbonylamino-*O*-(4-cyanobenzyl)-D-serine (3i). The title compound was prepared from **2** and 4-cyanobenzyl bromide by Method A (yield = 99%). ^{1}H NMR ($CDCl_3$) δ 7.47–7.37 (m, 2H), 7.33–7.24 (m, 2H), 4.64 (s, 2H), 4.50 (m, 1H), 4.03 (m, 1H), 3.79 (dd, 1H, J^1 = 3.6 Hz, J^2 = 9.3 Hz), 1.45 (s, 9H). MS m/z 319 (M^-−1).

5.4.11. *N*-*tert*-Butoxycarbonylamino-*O*-(2-cyanobenzyl)-D-serine (3j). The title compound was prepared from **2** and 2-cyanobenzyl bromide by Method A (yield = 77%). ^{1}H NMR ($CDCl_3$) δ 7.67–7.48 (m, 2H), 7.38 (m, 1H), 5.50 (m, 1H), 4.72 (s, 2H), 4.52 (m, 1H), 4.03 (m, 1H), 3.83 (dd, 1H, J^1 = 2.0 Hz, J^2 = 9.09 Hz), 3.77 (dd, 1H, J^1 = 3.4 Hz, J^2 = 9.5 Hz), 1.45 (s, 9H). MS m/z 319 (M^-−1).

5.4.12. *N*-*tert*-Butoxycarbonylamino-*O*-(2-trifluoromethylbenzyl)-D-serine (3k). The title compound was prepared from **2** and 2-trifluoromethylbenzyl bromide by Method

A (yield = 59%). ^{1}H NMR ($CDCl_3$) δ 8.59 (br s, 1H), 7.66–7.48 (m, 3H), 7.36 (m, 1H), 5.45 (d, 1H, J = 8.3 Hz), 4.72 (s, 2H), 4.53 (m, 1H), 4.00 (m, 1H), 3.78 (dd, 1H, J^1 = 3.4 Hz, J^2 = 9.5 Hz), 1.45 (s, 9H). MS *m/z* 362 (M^-−1).

5.4.13. *N-tert*-Butoxycarbonylamino-*O*-(2-methylbenzyl)-D-serine (3l). The title compound was prepared from **2** and 2-methylbenzyl bromide by Method A (yield = 64%). ^{1}H NMR (DMSO-d_6) δ 7.37–7.10 (m, 4H), 6.90 (d, 1H, J = 8.3 Hz), 4.47 (s, 2H), 4.19 (m, 1H), 3.67 (m, 2H), 2.25 (s, 3H), 1.38 (s, 9H). MS *m/z* 332 (M^++Na).

5.4.14. *N-tert*-Butoxycarbonylamino-*O*-(2-carboxamidobenzyl)-D-serine (3m). Compound **2** was alkylated with 2-cyanobenzyl bromide by Method A (yield = 97%). Subsequently, sodium perborate tetrahydrate (1.44 g, 9.4 mmol) was dissolved in water (10 ml), the nitrile (1 g, 3.12 mmol) and MeOH (10 ml) were added and stirred for 72 h. MeOH was evaporated and the residue was extracted with $CHCl_3$, the organic layer was dried (Na_2SO_4) and concentrated. Chromatography on silica (CH_2Cl_2/MeOH 10:1) yielded the title compound in 47% yield. ^{1}H NMR ($CDCl_3$) δ 7.78–7.27 (m, 4H), 5.60 (d, 1H, J = 8.3 Hz), 4.62 (m, 2H), 4.46 (m, 1H), 3.98 (m, 1H), 3.76 (m, 1H), 1.43 (s, 9H). MS *m/z* 361 (M^++Na).

5.4.15. *N-tert*-Butoxycarbonylamino-*O*-(2,3-dichlorobenzyl)-D-serine (3n). The title compound was prepared from **2** and 2,3-dichlorobenzyl bromide by Method A (yield = 36%). ^{1}H NMR (DMSO-d_6) δ 7.60 (m, 1H), 7.37 (m, 2H), 6.98 (d, 1H, J = 8.3 Hz), 4.53 (m, 2H), 4.22 (m, 1H), 3.74 (m, 2H), 1.37 (s, 9H). MS *m/z* 386 (M^++Na).

5.4.16. *N-tert*-Butoxycarbonylamino-*O*-(2,3-difluorobenzyl)-D-serine (3o). The title compound was prepared from **2** and 2,3-difluorobenzyl bromide by Method A (yield = 44%). ^{1}H NMR ($CDCl_3$) δ 7.22–7.00 (m, 3H), 5.39 (d, 1H, J = 7.3 Hz), 4.78 and 4.62 (2m, 2H), 4.48 (m, 1H), 3.97 (dd, 1H, J^1 = 2.0 Hz, J^2 = 9.3 Hz), 3.77 (dd, 1H, J^1 = 3.4 Hz, J^2 = 9.3 Hz), 1.45 (s, 9H). MS *m/z* 330 (M^-−1).

5.4.17. *N-tert*-Butoxycarbonylamino-*O*-(2,4-difluorobenzyl)-D-serine (3p). The title compound was prepared from **2** and 2,4-difluorobenzyl bromide by Method A (yield = 86%). ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 1H), 6.90–6.73 (m, 2H), 5.38 (d, 1H, J = 7.9 Hz), 4.56 (s, 2H), 4.47 (m, 1H), 3.95 (dd, 1H, J^1 = 2.4 Hz J^2 = 9.3 Hz), 3.74 (dd, 1H, J^1 = 3.6 Hz J^2 = 9.3 Hz), 1.45 (s, 9H). MS *m/z* 330 (M^-−1).

5.4.18. *N-tert*-Butoxycarbonylamino-*O*-(2,5-difluorobenzyl)-D-serine (3q). The title compound was prepared from **2** and 2,5-difluorobenzyl bromide by Method A (yield = 58%). ^{1}H NMR ($CDCl_3$) δ 9.12 (br s, 1H), 7.12–6.87 (m, 3H), 5.44 (d, 1H, J = 8.3 Hz), 4.57 (s, 2H), 4.51 (m, 1H), 3.98 (m, 1H), 3.78 (dd, 1H, J^1 = 3.4 Hz, J^2 = 9.3 Hz), 1.45 (s, 9H). MS *m/z* 330 (M^-−1).

5.4.19. *N-tert*-Butoxycarbonylamino-*O*-(3,5-difluorobenzyl)-D-serine (3r). The title compound was prepared from **2** and 3,5-difluorobenzyl bromide by Method A (yield = 57%). ^{1}H NMR ($CDCl_3$) δ 9.75 (br s, 1H), 6.88–6.65 (m, 3H), 5.44 (d, 1H, J = 8.3 Hz), 4.52 (m, 3H), 3.95 (m, 1H), 3.75 (dd, 1H, J^1 = 3.4 Hz, J^2 = 9.3 Hz), 1.45 (s, 9H). MS *m/z* 330 (M^-−1).

5.4.20. *N-tert*-Butoxycarbonylamino-*O*-(2,6-difluorobenzyl)-D-serine (3s). The title compound was prepared from **2** and 2,6-difluorobenzyl bromide by Method A (yield = 75%). ^{1}H NMR ($CDCl_3$) δ 8.15 (br s, 1H), 7.27 (m, 1H), 6.93–6.82 (m, 2H), 5.37 (d, 1H, J = 8.3 Hz), 4.63 (s, 2H), 4.44 (m, 1H), 3.94 (m, 1H), 3.74 (m, 1H), 1.45 (s, 9H). MS *m/z* 330 (M^-−1).

5.4.21. *N-tert*-Butoxycarbonylamino-*O*-(2,6-dichlorobenzyl)-D-serine (3t). The title compound was prepared from **2** and 2,6-dichlorobenzyl bromide by Method A (yield = 69%). ^{1}H NMR ($CDCl_3$) δ 7.33–7.15 (m, 3H), 5.40 (d, 1H, J = 8.5 Hz), 4.82 (s, 2H), 4.43 (m, 1H), 3.98 (m, 1H), 3.77 (dd, 1H, J^1 = 3.6 Hz, J^2 = 9.3 Hz), 1.45 (s, 9H). MS *m/z* 386 (M^++Na).

5.4.22. *N-tert*-Butoxycarbonylamino-*O*-(2,4,5-trifluorobenzyl)-D-serine (3u). The title compound was prepared from **2** and 2,4,5-trifluorobenzyl bromide by Method A (yield = 90%). ^{1}H NMR (DMSO-d_6) δ 7.60–7.48 (m, 2H), 7.03 (d, 1H, J = 8.3 Hz), 4.50 (s, 2H), 4.20 (m, 1H), 3.69 (m, 2H), 1.38 (s, 9H). MS *m/z* 348 (M^-−1).

5.4.23. *N-tert*-Butoxycarbonylamino-*O*-(2,3,5-trifluorobenzyl)-D-serine (3v). The title compound was prepared from **2** and 2,3,5-trifluorobenzyl bromide by Method A (yield = 98%). ^{1}H NMR (DMSO-d_6) δ 7.52 (m, 1H), 7.18 (m, 1H), 6.83 (d, 1H, J = 8.3 Hz), 4.61 (s, 2H), 4.17 (m, 1H), 3.71 (m, 2H), 1.37 (s, 9H). MS *m/z* 372 (M^++Na).

5.4.24. *N-tert*-Butoxycarbonylamino-*O*-(2,3,6-trifluorobenzyl)-D-serine (3w). The title compound was prepared from **2** and 2,3,6-trifluorobenzyl bromide by Method A (yield = 84%). ^{1}H NMR (DMSO-d_6) δ 7.53 (m, 1H), 7.18 (m, 1H), 6.85 (d, 1H, J = 8.3 Hz), 4.63 (s, 2H), 4.17 (m, 1H), 3.71(m, 2H), 1.37 (s, 9H). MS *m/z* 372 (M^++Na).

5.4.25. *N-tert*-Butoxycarbonylamino-*O*-(2-chloro-3,6-difluorobenzyl)-D-serine (3x). The title compound was prepared from **2** and 2-chloro-3,6-difluorobenzyl bromide by Method A (yield = 69%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 1H), 7.24 (m, 1H), 6.85 (d, 1H, J = 8.3 Hz), 4.61 (s, 2H), 4.15 (m, 1H), 3.69 (m, 2H), 1.37 (s, 9H). MS *m/z* 388 (M^++Na).

5.4.26. *N-tert*-Butoxycarbonylamino-*O*-(2-chloro-6-fluorobenzyl)-D-serine (3y). The title compound was prepared from **2** and 2-chloro-6-fluorobenzyl bromide by Method A (yield = 41%). ^{1}H NMR ($CDCl_3$) δ 7.50 (br s, 1H), 7.28–7.16 (m, 2H), 6.99 (m, 1H), 5.40 (d, 1H, J = 8.1 Hz), 4.70 (s, 2H), 4.45 (m, 1H), 3.96 (m, 1H), 3.74 (m, 1H), 1.44 (s, 9H). MS *m/z* 346 (M^-−1).

5.4.27. *N*-*tert*-Butoxycarbonylamino-*O*-(2,6-difluoro-3-methylbenzyl)-D-serine (3z). The title compound was prepared from **2** and 2,6-difluoro-3-methylbenzyl bromide by Method A (yield = 98%). ^{1}H NMR ($CDCl_3$) δ 10.2 (br s, 1H), 7.09 (m, 1H), 6.77 (m, 1H), 5.38 (d, 1H, J = 8.3 Hz), 4.62 (s, 2H), 4.44 (m, 1H), 3.94 (m, 1H), 3.73 (dd, 1H, J^1 = 3.6 Hz, J^2 = 9.5 Hz), 2.23 (s, 3H), 1.43 (s, 9H). MS *m*/*z* 368 (M^++Na).

5.4.28. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(4-fluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9a). The title compound was prepared from **3a** as described in Methods B and C (yield over all steps = 45%). ^{1}H NMR ($CDCl_3$) δ 7.45 (d, 2H, J = 8.4 Hz), 7.38 (d, 2H, J = 8.4 Hz), 7.22 (dd, 2H, J^1 = 8.4 Hz, J^2 = 5.5 Hz), 7.02 (dd, 2H, J^1 = 8.4 Hz, J^2 = 8.7 Hz), 4.89 (dd, 1H, J^1 = 6.8 Hz, J^2 = 14.0 Hz), 4.41 (s, 2H), 4.35 (m, 2H), 3.46 (m, 2H), 3.20 (m, 2H), 1.57–1.23 (m, 12H), 0.89 (t, 3H). MS *m*/*z* 595 (MH^+).

5.4.29. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(3-fluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9b). The title compound was prepared from **3b** as described in Methods B and C (yield = 55%). ^{1}H NMR (DMSO-d_6) δ 7.56 (d, 2H, J = 8.5 Hz), 7.43 (d, 2H, J = 8.5 Hz), 7.40 (m, 1H), 7.11 (m, 3H), 4.67 (dd, 1H, J^1 = 5.1 Hz, J^2 = 8.3 Hz), 4.54 (d, 1H, J = 16.2 Hz), 4.43 (s, 2H), 4.04 (d, 1H, J = 16.2 Hz), 3.36 (dd, 1H, J^1 = 8.7 Hz, J^2 = 9.7 Hz), 3.08 (m, 3H), 1.46 (s, 3H), 1.44 (s, 3H), 1.41 (s, 3H), 1.40 (s, 3H), 0.93 (t, 3H). MS *m*/*z* 595 (MH^+).

5.4.30. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-fluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9c). The title compound was prepared from **3c** as described in Methods B and C (yield = 52%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H), 7.42 (m, 2H), 7.38 (m, 2H), 7.20 (m, 2H), 4.64 (m, 1H), 4.51 (m, 3H), 4.03 (d, 1H, J = 16.2 Hz), 3.37 (m, 1H), 3.08 (m, 3H), 1.52–1.36 (m, 12H), 0.93 (m, 3H). MS *m*/*z* 595 (MH^+).

5.4.31. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(4-chlorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9d). The title compound was prepared from **3d** as described in Methods B and C (yield = 38%). ^{1}H NMR (DMSO-d_6) δ 7.57 (m, 2H), 7.42 (m, 4H), 7.30 (m, 2H), 4.63 (m, 1H), 4.53 (d, 1H, J = 16.2 Hz), 4.40 (s, 2H), 4.04 (d, 1H, J = 16.2 Hz), 3.35 (m, 1H), 3.06 (m, 3H), 1.53–1.37 (m, 12H), 0.93 (m, 3H). MS *m*/*z* 611 (MH^+).

5.4.32. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(4-trifluoromethylphenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9e). The title compound was prepared from **3e** as described in Methods B and C (yield = 32%). ^{1}H NMR (DMSO-d_6) δ 7.72 (m, 2H), 7.56 (m, 2H), 7.52–7.41 (m, 4H), 4.68 (m, 1H), 4.53 (m, 3H), 4.07 (d, 1H, J = 16.2 Hz), 3.37 (m, 1H), 3.08 (m, 3H), 1.52–1.37 (m, 12H), 0.93 (m, 3H). MS *m*/*z* 645 (MH^+).

5.4.33. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(4-trifluoromethoxyphenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9f). The title compound was prepared from **3f** as described in Methods B and C (yield = 16%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H), 7.46–7.32 (m, 6H), 4.65 (m, 1H), 4.52 (d, 1H, J = 16.1 Hz), 4.43 (s, 2H), 4.05 (d, 1H, J = 16.1 Hz), 3.35 (m, 1H), 3.05 (m, 3H), 1.42 (m, 12H), 0.92 (m, 3H). MS *m*/*z* 661 (MH^+).

5.4.34. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(4-chloro-2-fluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9g). The title compound was prepared from **3g** as described in Methods B and C (yield = 25%). ^{1}H NMR (DMSO-d_6) δ 7.55 (m, 2H), 7.47–7.27 (m, 5H), 4.65 (m, 1H), 4.48 (m, 3H), 4.06 (d, 1H, J = 16.1 Hz), 3.35 (m, 1H), 3.08 (m, 3H), 1.43 (m, 12H), 0.92 (m, 3H). MS *m*/*z* 629 (MH^+).

5.4.35. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-chloro-4-fluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9h). The title compound was prepared from **3h** as described in Methods B and C (yield = 28%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H), 7.43 (m, 4H), 7.23 (m, 1H), 4.64 (m, 1H), 4.55 (d, 1H, J = 16.1 Hz), 4.45 (s, 2H), 4.02 (d, 1H, J = 16.1 Hz), 3.38 (m, 1H), 3.07 (m, 3H), 1.43 (m, 12H), 0.95 (m, 3H). MS *m*/*z* 629 (MH^+).

5.4.36. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(4-cyanophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9i). The title compound was prepared from **3i** as described in Methods B and C (yield = 59%). ^{1}H NMR (DMSO-d_6) δ 8.27 (br s, 2H), 7.91–7.79 (m, 3H), 7.59–7.52 (m, 2H), 7.49–7.40 (m, 3H), 4.67 (m, 1H), 4.56 (d, 1H, J = 15.9 Hz), 4.51 (s, 2H), 4.06 (d, 1H, J = 15.9 Hz), 3.36 (m, 1H), 3.18–3.01 (m, 3H), 1.53–1.32 (m, 12H), 0.93 (m, 3H). MS *m*/*z* 600 (M^-−H).

5.4.37. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-cyanophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9j). The title compound was prepared from **3j** as described in Methods B and C (yield = 14%). ^{1}H NMR (DMSO-d_6) δ 7.87–7.27 (m, 8H), 4.65 (m, 1H), 4.59 (s, 2H), 4.43 (d, 1H, J = 16.1 Hz), 4.15 (d, 1H, J = 16.1 Hz), 3.40 (m, 2H), 3.09 (m, 2H), 1.43–1.10 (m, 12H), 0.85 (m, 3H). MS *m*/*z* 602 (MH^+).

5.4.38. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-trifluoromethylphenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9k). The title compound was prepared from **3k** as described in

Methods B and C (yield = 58%). ^{1}H NMR (DMSO-d_6) δ 7.74–7.27 (m, 8H), 4.65 (m, 1H), 4.59 (s, 2H), 4.45 (d, 1H, J = 6.0 Hz), 4.14 (d, 1H, J = 6.0 Hz), 3.38 (m, 2H), 3.19 (m, 2H), 1.43–1.11 (m, 12H), 0.85 (m, 3H). MS m/z 645 (MH^+).

5.4.39. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-methylphenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9l). The title compound was prepared from **3l** as described in Methods B and C (yield = 26%). ^{1}H NMR (DMSO-d_6) δ 7.57 (m, 2H), 7.44 (m, 2H), 7.18 (m, 4H), 4.66 (m, 1H), 4.53 (d, 1H, J = 16.2 Hz), 4.39 (s, 2H), 4.05 (d, 1H, J = 16.2 Hz), 3.33 (m, 1H), 3.04 (m, 3H), 2.24 (s, 3H), 1.43 (m, 6H), 1.39 (s, 6H), 0.94 (m, 3H). MS m/z 591 (MH^+).

5.4.40. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-carboxamidophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9m). The title compound was prepared from **3m** as described in Methods B and C (yield = 14%). ^{1}H NMR (DMSO-d_6) δ 7.58–7.29 (m, 8H), 4.69–4.52 (m, 4H), 4.01 (d, 1H, J = 16.1 Hz), 3.35 (m, 1H), 3.10 (m, 3H), 1.45 (m, 6H), 1.40 (m, 6H), 0.94 (m, 3H). MS m/z 620 (MH^+).

5.4.41. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,3-dichlorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9n). The title compound was prepared from **3n** as described in Methods B and C (yield = 34%). ^{1}H NMR (DMSO-d_6) δ 7.64–7.53 (m, 3H), 7.46–7.30 (m, 4H), 4.68 (m, 1H), 4.58 (m, 1H), 4.52 (s, 2H), 4.04 (d, 1H, J = 16.3 Hz), 3.40 (m, 1H), 3.09 (m, 3H), 1.43 (m, 12H), 0.95 (m, 3H). MS m/z 645 (MH^+).

5.4.42. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,3-difluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9o). The title compound was prepared from **3o** as described in Methods B and C (yield = 33%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H), 7.43 (m, 2H), 7.38 (m, 1H), 7.21 (m, 2H), 4.64 (m, 1H), 4.52 (m, 3H), 4.05 (d, 1H, J = 16.3 Hz), 3.38 (m, 1H), 3.10 (m, 3H), 1.52–1.38 (m, 12H), 0.93 (m, 3H). MS m/z 613 (MH^+).

5.4.43. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,4-difluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9p). The title compound was prepared from **3p** as described in Methods B and C (yield = 50%). ^{1}H NMR ($CDCl_3$) δ 7.48–7.22 (m, 5H), 6.82 (m, 2H), 4.89 (m, 2H), 4.47 (m, 2H), 4.28 (m, 1H), 3.53 (m, 2H), 3.22 (m, 2H), 1.61–1.38 (m, 12H), 0.87 (m, 3H). MS m/z 613 (MH^+).

5.4.44. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,5-difluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9q). The title compound was prepared from **3q** as described in Methods B and C (yield = 40%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H), 7.43 (m, 2H), 7.22 (m, 3H), 4.65 (m, 1H), 4.51 (m, 3H), 4.05 (d, 1H, J = 16.3 Hz), 3.38 (m, 1H), 3.10 (m, 3H), 1.52–1.36 (m, 12H), 0.93 (m, 3H). MS m/z 613 (MH^+).

5.4.45. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(3,5-difluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9r). The title compound was prepared from **3r** as described in Methods B and C (yield = 36%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 2H), 7.43 (m, 2H), 7.13 (m, 1H), 6.68 (m, 2H), 4.66 (m, 1H), 4.48 (m, 3H), 4.04 (d, 1H, J = 16.2 Hz), 3.36 (m, 1H), 3.10 (m, 3H), 1.52–1.36 (m, 12H), 0.93 (m, 3H). MS m/z 613 (MH^+).

5.4.46. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,6-difluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9s). The title compound was prepared from **3s** as described in Methods B and C (yield = 27%). ^{1}H NMR (DMSO-d_6) δ 7.58–7.40 (m, 5H), 7.11 (m, 2H), 4.59 (m, 1H), 4.48 (m, 3H), 4.04 (d, 1H, J = 16.1 Hz), 3.37 (m, 1H), 3.17 (m, 1H), 3.04 (m, 2H), 1.50–1.32 (m, 12H), 0.89 (m, 3H). MS m/z 613 (MH^+).

5.4.47. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,6-dichlorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9t). The title compound was prepared from **3t** as described in Methods B and C (yield = 38%). ^{1}H NMR (DMSO-d_6) δ 7.58–7.24 (m, 7H), 4.62 (m, 3H), 4.41 (d, 1H, J = 16.0 Hz), 4.14 (d, 1H, J = 16.0 Hz), 3.41 (m, 2H), 3.06 (m, 2H), 1.38–1.10 (m, 12H), 0.82 (m, 3H). MS m/z 654 (MH^+).

5.4.48. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,4,5-trifluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9u). The title compound was prepared from **3u** as described in Methods B and C (yield = 19%). ^{1}H NMR (DMSO-d_6) δ 7.65–7.30 (m, 6H), 4.66 (m, 1H), 4.53 (d, 1H, J = 16.2 Hz), 4.42 (m, 2H), 4.07 (d, 1H, J = 16.2 Hz), 3.35 (m, 1H), 3.09 (m, 3H), 1.42 (m, 12H), 0.93 (m, 3H). MS m/z 631 (MH^+).

5.4.49. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,3,5-trifluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9v). The title compound was prepared from **3v** as described in Methods B and C (yield = 18%). ^{1}H NMR (DMSO-d_6) δ 7.60–7.30 (m, 5H), 7.18 (m, 1H), 4.66 (m, 1H), 4.60–4.45 (m, 3H), 4.06 (d, 1H, J = 16.1 Hz), 3.38 (m, 1H), 3.12 (m, 3H), 1.42 (m, 12H), 0.93 (m, 3H). MS m/z 631 (MH^+).

5.4.50. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,3,6-trifluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9w). The title compound was prepared from **3w** as described in Methods B and C (yield = 32%). ^{1}H NMR (DMSO-d_6) δ 7.56 (m, 3H), 7.44 (m, 2H), 7.18 (m, 1H), 4.61 (m, 1H), 4.50 (m,

3H), 4.07 (d, 1H, $J = 16.1$ Hz), 3.38 (m, 1H), 3.17 (m, 1H), 3.04 (m, 2H), 1.42 (m, 12H), 0.89 (m, 3H). MS *m/z* 631 (MH^+).

5.4.51. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-chloro-3,6-difluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9x). The title compound was prepared from **3x** as described in Methods B and C (yield = 32%). ^{1}H NMR (DMSO-d_6) δ 7.60–7.29 (m, 6H), 4.61 (m, 1H), 4.44 (s, 2H), 4.48 (d, 1H, $J = 16.2$ Hz), 4.07 (d, 1H, $J = 16.2$ Hz), 3.38 (m, 1H), 3.17 (m, 1H), 3.05 (m, 2H), 1.49–1.35 (m, 12H), 0.90 (m, 3H). MS *m/z* 647 (MH^+).

5.4.52. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2-chloro-6-fluorophenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9y). The title compound was prepared from **3y** as described in Methods B and C (yield = 50%). ^{1}H NMR (DMSO-d_6) δ 7.58 (d, 2H, $J = 8.5$ Hz), 7.50–7.20 (m, 5H), 4.61 (dd, 1H, $J^1 = 5.4$ Hz, $J^2 = 8.0$ Hz), 4.53 (s, 2H), 4.50 (d, 1H, $J = 16.1$ Hz), 4.05 (d, 1H, $J = 16.2$ Hz), 3.40 (dd, 1H, $J^1 = 9.4$ Hz, $J^2 = 8.9$ Hz), 3.14 (dd, 1H, $J^1 = 4.9$ Hz, $J^2 = 9.9$ Hz), 3.05 (m, 2H), 1.42 (m, 6H), 1.39 (s, 3H), 1.38 (s, 3H), 0.91 (t, 3H). MS *m/z* 629 (MH^+).

5.4.53. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(2,6-difluoro-3-methylphenyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (9z). The title compound was prepared from **3z** as described in Methods B and C (yield = 37%). ^{1}H NMR (DMSO-d_6) δ 7.54 (m, 2H), 7.41 (m, 2H), 7.31 (m, 1H), 6.99 (m, 1H), 4.59–4.45 (m, 4H), 4.04 (d, 1H, $J = 16.3$ Hz), 3.37 (m, 1H), 3.13 (m, 1H), 3.04 (m, 2H), 2.20 (s, 3H), 1.42 (m, 12H), 0.90 (m, 3H). MS *m/z* 627 (MH^+).

5.4.54. *N*-*tert*-Butoxycarbonylamino-*O*-pyridin-2-ylmethyl-D-serine (10a). The title compound was prepared from **2** and 2-bromomethyl-pyridine by Method A (yield = 62%). ^{1}H NMR ($CDCl_3$) δ 8.94 (br s, 2H), 8.60 (d, 1H, $J = 4.6$ Hz), 7.78 (m, 1H), 7.42 (d, 1H, $J = 7.9$ Hz), 7.30 (m, 1H), 5.72 (d, 1H, $J = 7.6$ Hz), 4.76 (m, 2H), 4.46 (m, 1H), 4.03 (dd, 1H, $J^1 = 3.4$ Hz, $J^2 = 10.0$ Hz), 3.88 (dd, 1H, $J^1 = 4.6$ Hz, $J^2 = 10.0$ Hz), 1.44 (s, 9H). MS *m/z* 295 ($M^- - 1$).

5.4.55. *N*-*tert*-Butoxycarbonylamino-*O*-pyridin-3-ylmethyl-D-serine (10b). The title compound was prepared from **2** and 3-bromomethyl-pyridine by Method A (yield = 67%). ^{1}H NMR ($CDCl_3$) δ 8.60 (br s, 1H), 8.42 (m, 2H), 7.90–7.70 (m, 1H), 7.40 (m, 1H), 7.30 (m, 1H), 5.61 (d, 1H, $J = 7.5$ Hz), 4.77–4.28 (m, 3H), 4.01 (dd, 1H, $J^1 = 2.4$ Hz, $J^2 = 9.2$ Hz), 3.82 (m, 1H), 1.44 (s, 9H). MS *m/z* 295 ($M^- - 1$).

5.4.56. *N*-*tert*-Butoxycarbonylamino-*O*-pyridin-4-ylmethyl-D-serine (10c). The title compound was prepared from **2** and 4-bromomethyl-pyridine by Method A (yield = 34%). ^{1}H NMR ($CDCl_3$) δ 9.57 (br s, 2H), 8.49 (m, 2H), 7.35 (m, 2H), 5.60 (d, 1H, $J = 7.5$ Hz), 4.80–4.42 (m, 3H), 4.02 (m, 1H), 3.88 (m, 1H), 1.44 (s, 9H). MS *m/z* 295 ($M^- - 1$).

5.4.57. *N*-*tert*-Butoxycarbonylamino-*O*-(3,5-dimethyl-isoxazol-4-ylmethyl)-D-serine (10d). The title compound was prepared from **2** and 4-bromomethyl-3,5-dimethyl-isoxazole by Method A (yield = 70%). ^{1}H NMR ($CDCl_3$) δ 8.04 (br s, 1H), 5.38 (d, 1H, $J = 8.6$ Hz), 4.47 (m, 1H), 4.30 (m, 2H), 3.85 (m, 1H), 3.68 (m, 1H), 2.37 (s, 3H), 2.21 (s, 3H), 1.44 (s, 9H). MS *m/z* 313 ($M^- - 1$).

5.4.58. *N*-*tert*-Butoxycarbonylamino-*O*-(2-methyl-thiazol-4-ylmethyl)-D-serine (10e). The title compound was prepared from **2** and 4-bromomethyl-2-methyl-thiazole by Method A (yield = 51%). ^{1}H NMR ($CDCl_3$) δ 9.30 (br s, 1H), 7.05 (m, 1H), 5.68 (d, 1H, $J = 8.1$ Hz), 4.63 (s, 2H), 4.47 (m, 1H), 4.02 (m, 1H), 3.78 (dd, 1H, $J^1 = 3.6$ Hz, $J^2 = 9.6$ Hz), 2.71 (s, 3H), 1.45 (s, 9H). MS *m/z* 315 ($M^- - 1$).

5.4.59. *N*-*tert*-Butoxycarbonylamino-*O*-thiazol-4-ylmethyl-D-serine (10f). The title compound was prepared from **2** and 4-bromomethyl-thiazole by Method A (yield = 41%). ^{1}H NMR ($CDCl_3$) δ 8.88 (br s, 2H), 7.31 (s, 1H), 5.65 (d, 1H, $J = 8.1$ Hz), 4.73 (m, 2H), 4.48 (m, 1H), 4.03 (m, 1H), 3.80 (dd, 1H, $J^1 = 3.6$ Hz, $J^2 = 9.7$ Hz), 1.44 (s, 9H). MS *m/z* 301 ($M^- - 1$).

5.4.60. *N*-*tert*-Butoxycarbonylamino-*O*-thien-3-ylmethyl-D-serine (10g). The title compound was prepared from **2** and 3-bromomethyl-thiophene by Method A (yield = 30%). ^{1}H NMR ($CDCl_3$) δ 8.83 (br s, 1H), 7.33–7.00 (m, 3H), 5.40 (d, 1H, $J = 8.1$ Hz), 4.72–4.53 (m, 2H), 4.47 (m, 1H), 3.91 (m, 1H), 3.70 (dd, 1H, $J^1 = 3.3$ Hz, $J^2 = 9.3$ Hz), 1.44 (s, 9H). MS *m/z* 300 ($M^- - 1$).

5.4.61. *N*-*tert*-Butoxycarbonylamino-*O*-thien-2-ylmethyl-D-serine (10h). The title compound was prepared from **2** and 2-bromomethyl-thiophene by Method A (yield = 45%). ^{1}H NMR ($CDCl_3$) δ 8.85 (br s, 1H), 7.28 (m, 1H), 6.95 (m, 2H), 5.40 (d, 1H, $J = 8.1$ Hz), 4.73 (m, 2H), 4.47 (m, 1H), 3.91 (m, 1H), 3.70 (dd, 1H, $J^1 = 3.3$ Hz, $J^2 = 9.3$ Hz), 1.44 (s, 9H). MS *m/z* 300 ($M^- - 1$).

5.4.62. *N*-*tert*-Butoxycarbonylamino-*O*-(1-phenylethyl)-D-serine (10i). The title compound was prepared from **2** and (1-bromoethyl)-benzene by Method A (yield = 50%). ^{1}H NMR (DMSO-d_6) δ 12.60 (br s, 1H), 7.30 (m, 5H), 6.85 (d, 1H, $J = 8.3$ Hz), 4.45 (m, 1H), 3.37 (m, 1H), 3.63 (m, 2H), 1.38 (s, 9H), 1.32 (d, 3H, $J = 6.4$ Hz). MS *m/z* 308 ($M^- - 1$).

5.4.63. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(pyridin-2-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11a). The title compound was prepared from **10a** as described in Methods B and C (yield over all steps = 30%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.73 (m, 1H), 8.35 (m, 1H), 7.79 (m, 2H), 7.56 (m, 2H), 7.43 (m, 2H), 4.72 (m, 3H), 4.44 (d, 1H, $J = 16.1$ Hz), 4.05 (d, 1H, $J = 16.1$ Hz), 3.48 (m, 1H), 3.27 (m, 1H), 3.09 (m, 2H), 1.53–1.37 (m, 12H), 0.95 (m, 3H). MS *m/z* 578 (MH^+).

5.4.64. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(pyridin-3-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11b). The title compound was prepared from **10b** as described in Methods B and C (yield = 30%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.82 (m, 1H), 8.74 (m, 1H), 8.36 (m, 1H), 7.78 (dd, 1H, J^1 = 5.8 Hz, J^2 = 7.9 Hz,), 7.58 (m, 2H), 7.43 (m, 2H), 4.66 (m, 3H), 4.52 (d, 1H, J = 16.2 Hz), 4.06 (d, 1H, J = 16.2 Hz), 3.43 (m, 1H), 3.23 (m, 1H), 3.08 (m, 2H), 1.53–1.37 (m, 12H), 0.93 (m, 3H). MS *m*/*z* 578 (MH^+).

5.4.65. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(pyridin-4-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11c). The title compound was prepared from **10c** as described in Methods B and C (yield = 54%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 8.85 (m, 2H), 7.87 (m, 2H), 7.57 (m, 2H), 7.44 (m, 2H), 4.75 (m, 3H), 4.53 (d, 1H, J = 16.2 Hz), 4.07 (d, 1H, J = 16.2 Hz), 3.48 (m, 1H), 3.26 (m, 1H), 3.10 (m, 2H), 1.53–1.38 (m, 12H), 0.95 (m, 3H). MS *m*/*z* 578 (MH^+).

5.4.66. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(3,5-dimethyl-isoxazol-4-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11d). The title compound was prepared from **10d** as described in Methods B and C (yield = 54%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.60 (d, 2 H, J = 8.5 Hz), 7.44 (d, 2H, J = 8.5 Hz), 4.60 (m, 1H), 4.48 (d, 1H, J = 16.2 Hz), 4.21 (s, 2H), 4.05 (d, 1H, J = 16.2 Hz), 3.48 (dd, 1H, J^1 = 8.8 Hz, J^2 = 9.5 Hz), 3.07 (m, 3H), 2.33 (s, 3H), 2.14 (s, 3H), 1.50–1.38 (m, 12H), 0.92 (t, 3H, J = 6.9 Hz). MS *m*/*z* 596 (MH^+).

5.4.67. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3- (2-methyl-thiazol-4-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11e). The title compound was prepared from **10e** as described in Methods B and C (yield = 57%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.56 (m, 2H), 7.43 (m, 2H), 7.34 (s, 1H), 4.64 (m, 1H), 4.54 (d, 1H, J = 16.2 Hz), 4.44 (s, 2H), 4.03 (d, 1H, J = 16.2 Hz), 3.38 (m, 1H), 3.20–3.09 (m, 3H), 2.65 (s, 3H), 1.44 (m, 6H), 1.40 (m, 6H), 0.93 (m, 3H). MS *m*/*z* 598 (MH^+).

5.4.68. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(thiazol-4-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11f). The title compound was prepared from **10f** as described in Methods B and C (yield = 52%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 9.74 (d, 1H, J = 2.0 Hz), 7.57 (m, 3H), 7.43 (d, 2H, J = 8.5 Hz), 4.70–4.48 (m, 4H), 4.04 (d, 1H, J = 16.2 Hz), 3.40 (m, 1H), 3.19 (m, 1H), 3.05 (m, 2H), 1.53–1.35 (4s, 12H), 0.93 (t, 3H, J = 6.9 Hz). MS *m*/*z* 584 (MH^+).

5.4.69. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(thien-3-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11g). The title compound was prepared from **10g** as described in Methods B and C (yield = 29%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.57 (d, 2H, J = 8.5 Hz), 7.51 (dd, 1H, J^1 = 3.0 Hz, J^2 = 4.9 Hz), 7.43 (d, 2H, J = 8.5 Hz), 7.36 (m, 1H), 7.02 (dd, 1H, J^1 = 1.0 Hz, J^2 = 4.9 Hz), 4.62 (m, 1H), 4.55 (d, 1H, J = 16.2 Hz), 4.40 (s, 2H), 4.02 (d, 1H, J = 16.2 Hz), 3.32 (dd, 1H, dd, 1H, J^1 = 8.9 Hz, J^2 = 9.9 Hz), 3.03 (m, 3H), 1.45 (s, 3H), 1.43 (s, 3H), 1.40 (m, 6H), 0.92 (t, 3H, J = 6.8 Hz). MS *m*/*z* 583 (MH^+).

5.4.70. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(thien-2-yl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11h). The title compound was prepared from **10h** as described in Methods B and C (yield over all steps = 6%). ^{1}H NMR ($CDCl_3$) δ 7.44 (m, 2H), 7.39 (m, 2H), 7.28 (m, 1H), 6.95 (m, 2H), 4.88 (m, 1H), 4.61 (m, 3H), 4.16 (d, 1H, J = 16.2 Hz), 3.49 (m, 2H), 3.28 (m, 1H), 3.14 (m, 1H), 1.52 (s, 3H), 1.47 (s, 3H), 1.31 (s, 3H), 1.28 (s, 3H), 0.89 (m, 3H). MS *m*/*z* 583 (MH^+).

5.4.71. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-(1-phenylethyl)methoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (11i and 11j). The racemic compound **10i** was coupled with **4**[10] as described in Method B, but running the Boc-deprotection with TFA for only 1 h. The product (68 mg, 0.13 mmol) was then added to a solution of *N*-FMOC-α-amino-isobutyric acid (44 mg, 0.13 mmol), TBTU (52 mg, 0.16 mmol), and NEt_3 (23 μl, 0.16 mmol) in CH_2Cl_2 (10 ml) and the mixture was stirred overnight at rt. The solution was extracted with water and the organic layer was separated, dried over Na_2SO_4 and concentrated. The residue was dissolved in DMF (2 ml) and ethylamine (1 ml), left for 2 h at rt and was evaporated. Purification by RP-HPLC (YMC Combi Prep ODS A 5 μm 120A 20× 50 mm, CH_3CN–0.1% TFA in water) yielded the title compound as a mixture of isomers which was separated by HPLC (Chiralpak AD, hexane–0.05%TFA/isopropyl acetate). Yield over all steps = 5%. The NMR spectrum for both isomers is identical. ^{1}H NMR ($CDCl_3$) δ 7.95 (m, 1H), 7.40–7.12 (m, 9H), 4.79 (m, 1H), 4.50 (m, 2H), 4.29 (m, 1H), 4.15 (m, 1H), 3.26 (m, 2H), 3.14 (m, 2H), 1.46 (m, 6H), 1.29 (m, 3H), 1.22 (m, 6H), 0.84 (m, 3H). MS *m*/*z* 591 (MH^+).

5.4.72. (D)-*N*-Acetyl-2-amino-5-phenyl-pentanoic acid (14a). A solution of sodium ethoxide was generated by the addition of sodium metal (52.9 g, 2.30 mol) over 3 h to EtOH (1500 ml). To the sodium ethoxide solution at rt was added a solution of diethylacetamidomalonate (**12**, 499.75 g, 2.30 mol) dissolved in EtOH (225 ml). The reaction mixture was stirred for 1.5 h at rt, 1-bromo-3-phenylpropane (458 g, 2.30 mol) was added over 15 min and the mixture was refluxed for 16 h. The mixture was concentrated to dryness and the residue partitioned between ethyl acetate (500 ml) and water (1500 ml). The organic layers were combined, washed with satd NaCl solution, dried over Na_2SO_4 and evaporated to yield 2-acetylamino-2-(3-phenylpropyl)-malonic acid diethyl ester (**13a**) (752 g, 98%).

A slurry of **13a** (249 g, 0.74 mol) and 2.5 M NaOH was heated at 100 °C for 3 h. The mixture was cooled to

30 °C and the pH adjusted to 5.0 using concd HCl. The solution was heated to 100 °C and the pH was held at 5.0 using concd HCl until the reaction was complete. The hot solution was filtered through diatomaceous earth. The filtrate was cooled to 5–10 °C and the pH adjusted to 1.0 using concd HCl. The resulting slurry was stirred for 1 h at 5 °C, filtered and dried in vacuum to give 160 g (92%) of (DL)-*N*-acetyl-2-amino-5-phenylpentanoic acid. A solution of this racemic mixture (160 g, 0.68 mol), $CoCl_2$ (0.40 g), 2 M KCl solution (340 ml, 0.68 mol) and water (2900 ml) was adjusted to pH 8.0 using 2 M KOH. Acylase I (*Aspergillus melleus*, 14.40 g) was added and the mixture was stirred for 24 h at 40 °C, maintaining a pH of 8.0 by addition of 2 M KOH solution. The resulting slurry was filtered, and the filtrate adjusted to pH 2.0 giving a thick slurry. The product was isolated by filtration, washed with hexane (730 ml) and dried in vacuum at 50 °C to yield **14a** (68.9 g, 43%). ^{1}H NMR (DMSO-d_6) δ 12.39 (br s, 1H), 8.02 (d, 1H), 7.30–7.16 (m, 5H), 4.24 (m, 1H), 2.59 (m, 2H), 1.86 (s, 3H), 1.74–1.59 (m, 4H). MS *m*/*z* 236 (MH^+).

5.4.73. 2-(*tert*-Butoxycarbonylamino)-5-phenyl-D-pentanoic acid (15a). A solution of **14a** (188.5 g, 0.80 mol) in EtOH (535 ml) and concd HCl (318 ml, 3.80 mol) was warmed to 85 °C for 22 h. Water was azeotropically distilled from the reaction by continuous addition and distillation of EtOH (8000 ml). The EtOH was azeotropically distilled from the reaction by continuous addition and distillation of ethyl acetate (2000 ml). The solution was then stirred at 0 °C for 1 h, the crystallised product was filtered and dried in vacuum at 40 °C to give (D)-2-amino-5-phenylpentanoic acid, ethyl ester hydrochloride (199 g, 96%).

To a slurry of (D)-2-amino-5-phenylpentanoic acid, ethyl ester hydrochloride (10 g, 38.3 mmol) in THF (400 ml) were added triethylamine (10.7 ml, 76 mmol) and di-*tert*-butyl-dicarbonate (10.9 g, 50 mmol), the mixture was stirred for 18 h at rt, evaporated and dissolved in CH_2Cl_2. After extraction with 0.1 M citric acid, the organic layer was dried (Na_2SO_4) and concentrated. The crude product was suspended in 2.5 M NaOH and heated to 60 °C for 5 h. The mixture was cooled to rt, neutralised with concd HCl and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and concentrated to yield **15a** (7.30 g, 65%). ^{1}H NMR (DMSO-d_6) δ 12.4 (br s, 1H), 7.42 (d, 1H, J = 7.5 Hz), 7.30–7.08 (m, 5H), 4.27 (m, 1H), 2.58 (m, 2H), 1.57–1.75 (m, 4H), 1.35 (s, 9H). MS *m*/*z* 316 (M^++Na).

5.4.74. 2-(*tert*-Butoxycarbonylamino)-5-(4-fluorophenyl)-D-pentanoic acid (15b). The title compound was prepared from 1-bromo-3-(4-fluorophenyl)propane as described for **14a** and **15a** (total yield = 25%). ^{1}H NMR ($CDCl_3$) δ 8.87 (br s, 1H), 7.32–7.03 (m, 4H), 4.99 (d, 1H, J = 7.5 Hz), 4.35 (m, 1H), 2.66 (m, 2H), 1.95–1.60 (m, 4H), 1.44 (s, 9H). MS *m*/*z* 334 (M^++Na).

5.4.75. 2-(*tert*-Butoxycarbonylamino)-5-(3,5-difluorophenyl)-D-pentanoic acid (15c). The title compound was prepared from 1-bromo-3-(3,5-difluorophenyl)propane as described for **14a** and **15a** (total yield = 28%). ^{1}H NMR ($CDCl_3$) δ 8.90 (br s, 1H), 6.65–6.54 (m, 3H), 5.01 (d, 1H, J = 7.5 Hz), 4.36 (m, 1H), 2.63 (m, 2H), 1.98–1.60 (m, 4H), 1.44 (s, 9H). MS *m*/*z* 352 (M^++Na).

5.4.76. 2-(*tert*-Butoxycarbonylamino)-5-(2,6-difluorophenyl)-D-pentanoic acid (15d). The title compound was prepared from 1-bromo-3-(2,6-difluorophenyl)propane as described for **14a** and **15a** (total yield = 17%). ^{1}H NMR ($CDCl_3$) δ 8.93 (br s, 1H), 7.29 (m, 1H), 7.10–6-99 (m, 2H), 5.05 (d, 1H, J = 7.5 Hz), 4.38 (m, 1H), 2.65 (m, 2H), 2-08–1.67 (m, 4H), 1.42 (s, 9H). MS *m*/*z* 352 (M^++Na).

5.4.77. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-5-phenylpentanoic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (16a). The title compound was prepared from **15a** as described in Methods B and C (yield = 91%). ^{1}H NMR (DMSO-d_6) δ 8.39 (d, 1H, J = 8.0 Hz), 8.18 (br s, 3H), 7.86 (s, 1H), 7.52 (d, 2H, J = 8.6 Hz), 7.40 (d, 2H, J = 8.6 Hz), 7.29 (m, 2H), 7.22–7.12 (m, 3H), 4.64 (d, 1H, J = 16.3 Hz), 4.35 (ddd, 1H), 3.92 (d, 1H, J = 16.4 Hz), 2.91 (m, 2H), 2.48 (m, 2H), 1.50 (m, 2H), 1.46, 1.43, 1.41, 1.39 (4s, 12H), 1.28 (m, 2H), 0.95 (t, 3H, J = 6.9 Hz). MS *m*/*z* 576 (M^++H).

5.4.78. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-5-(4-fluorophenyl)-pentanoic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (16b). The title compound was prepared from **15b** as described in Methods B and C (yield = 15%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.52 (m, 2H), 7.40 (m, 2H), 7.20–7.05 (m, 4H), 4.51 (d, 1H, J = 16.4 Hz), 4.34 (m, 1H), 4.07 (d, 1H, J = 16.4 Hz), 2.96 (m, 2H), 2.50 (d, 2H), 2.00 (m, 2H), 1.45 (m, 2H), 1.39 (s, 6H), 1.13 (2s, 6H), 0.87 (t, 3H). MS *m*/*z* 594 (M^++H).

5.4.79. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-5-(3,5-difluorophenyl)-pentanoic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (16c). The title compound was prepared from **15c** as described in Methods B and C (yield = 43%). ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 1H, J = 9.0 Hz), 7.46–7.35 (m, 4H), 6.70–6.59 (m, 3H), 4.65–4.52 (m, 2H), 4.20 (d, 1H, J = 15.7 Hz), 3.18 (m, 1H), 2.97 (m, 1H), 2.57 (m, 2H), 1.59 (m, 2H), 1.57 (2s, 6H), 1.45–1.23 (m, 8H), 0.91 (t, 3H). MS *m*/*z* 612 (M^++H).

5.4.80. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-5-(2,6-difluorophenyl)- pentanoic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (16d). The title compound was prepared from **15d** as described in Methods B and C (yield = 33%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.50 (m, 2H), 7.40 (m, 2H), 7.29 (m, 1H), 7.04 (m, 2H), 4.51 (d, 1H, J = 16.3 Hz), 4.34 (m, 1H), 4.07 (d, 1H, J = 16.3 Hz), 2.99 (m, 2H), 2.55 (m, 2H), 2.05 (m, 2H), 1.44 (m, 2H), 1.39 (2s, 6H), 1.13 (2s, 6H), 0.85 (t, 3H). MS *m*/*z* 612 (M^++H).

5.4.81. 2-(*R*)-3-(*R*)-2-(2-Amino-2-methylpropionylamino)-3-benzyloxy-3-methyl- propionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (18). The title compound was prepared from *N*-Boc-*O*-benzyl-D-allo-threonine (**17**) as described in Methods B and C (yield = 18%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.62 (m, 2H), 7.48 (m, 2H), 7.38–7.24 (m, 5H), 4.63 (m, 1H), 4.53 (m, 2H), 4.41 (m, 1H), 4.05 (d, 1H, J = 16.3 Hz), 3.86 (m, 1H), 3.39 (m, 1H), 3.01 (m, 1H), 1.59–1.28 (m, 12H), 1.07 (d, 3H, J = 6.0 Hz), 0.75 (m, 3H). MS m/z 591 (MH^+).

5.4.82. *N*-Boc-*N*-methyl-*O*-benzyl-D-serine (21). A suspension of *N*-Boc-*O*-benzyl-D-serine (**19**, 1.95 g, 6.59 mmol), *p*-toluenesulfonic acid (600 mg, 3.16 mmol) and paraformaldehyde (1.04 g, 34.4 mmol) was stirred at 100 °C for 50 min. The solution was cooled to rt, diluted with ethyl acetate and extracted with water and $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated to yield **20** as a crystalline solid (884 mg, 44%). The product (884 mg, 2.88 mmol) was dissolved in chloroform (15 ml), triethylsilane (2.5 ml, 15.7 mmol) and trifluoroacetic acid (10 ml) were added and the solution was stirred at rt for 6 h. The mixture was concentrated, dissolved in isohexane and extracted with satd $NaHCO_3$ solution. The pH was then adjusted to 3.0 using 10% $KHSO_4$, and the aqueous layer, was extracted with ethyl acetate. After concentration of the aqueous layer the crude product was suspended in THF (60 ml), NEt_3 (5 ml, 35.6 mmol) and di-*tert*-butyl-dicarbonate (825 mg, 3.8 mmol) were added and the mixture was stirred overnight at rt. The solvent was evaporated, the residue was dissolved in *tert*-butyl-methyl ether and extracted with water. After acidification with 10% citric acid, the aqueous layer was extracted with ethyl acetate, the organic layer was dried (Na_2SO_4) and concentrated to yield the title compound as an amorphous solid (483 mg, 54%). ^{1}H NMR ($CDCl_3$) δ 9.66 (br s, 1H), 7.37–7.28 (m, 5H), 4.95 (m, 1H), 4.55 (m, 2H), 3.90 (m, 2H), 2.94 (s, 3H), 1.44 (s, 9H). MS m/z 308 ($M^- - 1$).

5.4.83. 2-(*R*)-2-[2-(*R*)-2-Aminopropionyl(*N*-methylamino)]-3-benzyloxypropionic acid *N*-(5-(4-chloro phenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (22) and 2-(*R*)-2-[2-(*S*)-2-Aminopropionyl(*N*-methylamino)]-3-benzyloxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (23). The title compounds were prepared as described in Methods B and C using **21** and *N*-Boc-D-alanine to obtain **22** (yield = 90%) or **21** and *N*-Boc-L-alanine to obtain **23** (yield = 98%). The NMR spectra for **22** and **23** are identical. ^{1}H NMR ($CDCl_3$) δ 7.46-7.23 (m, 9H), 5.43 (m, 1H), 4.61–4.36 (m, 3H), 4.18 (m, 1H), 3.83–3.62 (m, 2H), 3.45 (m, 1H), 3.27 (m, 1H), 3.07 (m, 1H), 2.93 (s, 3H), 1.52–1.44 (m, 6H), 1.18 (m, 3H), 0.86 (m, 3H). MS m/z 576 ($M^- - 1$).

5.4.84. 2-(*R*)-2-(2-Amino-3-fluoro-2-fluoromethylpropionylamino)-3-(2,6- difluorophenyl)methoxy propionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (24). To a suspension of methyl 2-amino-3-fluoro-2-fluoromethyl-propionate hydrochloride[9] (250 mg, 1.32 mmol) in acetonitrile (10 ml) was added $Me_4NOH{\cdot}5H_2O$ (400 mg, 2.20 mmol). The mixture was stirred at rt under Ar for 30 min and then di-*tert*-butyl-dicarbonate (432 mg, 1.98 mmol) was added. The mixture was stirred for 48 h, the solvent was evaporated and the residue was partitioned between water and diethyl ether. The aqueous layer was washed with ether, acidified with solid citric acid to pH 3–4 and extracted with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated to yield *N*-Boc-3-fluoro-2-fluoromethyl-alanine (294 mg, 92%) as a white solid.

The title compound was prepared from **6s** and *N*-Boc-3-fluoro-2-fluoromethyl-alanine as described in Method C (yield = 56%). ^{1}H NMR (DMSO-d_6) δ 9.01 (m, 3H), 7.86 (s, 1H), 7.59–7.39 (m, 5H), 7.12 (m, 2H), 5.13–4.62 (m, 5H), 4.48 (m, 3H), 4.09 (d, 1H, J = 16.0 Hz), 3.29–3.16 (m, 2H), 3.04 (m, 2H), 1.37 (s, 6H), 0.87 (t, 3H). MS m/z 650 (MH^+).

5.4.85. 2-(*R*)-2-((1-Amino-cyclopropanecarbonyl)-amino)-3-phenylmethoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (25). The title compound was prepared from **6** (X = H) and 1-(Boc-amino)cyclopropanecarboxylic acid as described in Method C (yield = 38%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.54 (m, 2H), 7.42 (m, 2H), 7.38–7.23 (m, 5H), 4.64 (m, 1H), 4.43 (m, 3H), 4.12 (d, 1H, J = 16.2 Hz), 3.34 (m, 2H), 3.04 (m, 2H), 1.39 (m, 6H), 1.04 (m, 2H), 0.85 (m, 2H), 0.79 (m, 3H). MS m/z 575 (MH^+).

5.4.86. 2-(*R*)-2-(2-(*R*)-2-Amino-2-methyl-3-hydroxypropionylamino)-3- phenylmethoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (26). The title compound was prepared from **6** (X = H) and *N*-Boc-α-methyl-D-serine as described in Method C (yield = 71%). ^{1}H NMR (DMSO-d_6) δ 8.52 (d, 1H, J = 7.9 Hz), 8.12 (br s, 3H), 7.87 (br s, 1H), 7.56 (m, 2H), 7.45 (m, 2H), 7.39–7.25 (m, 5H), 5.58 (m, 1H), 4.71 (m, 1H), 4.52 (d, 1H, J = 16.0 Hz), 4.41 (s, 2H), 4.06 (d, 1H, J = 16.0 Hz), 3.72 (m, 1H), 3.62 (m, 1H), 3.35 (m, 1H), 3.13 (m, 1H), 3.05 (m, 2H), 1.47–1.32 (m, 9H), 0.90 (m, 3H). MS m/z 593 (MH^+).

5.4.87. 2-(*R*)-2-(2-(*S*)-2-Amino-2-methyl-3-hydroxypropionylamino)-3- phenylmethoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (27). The title compound was prepared from **6** (X = H) and *N*-Boc-α-methyl-L-serine as described in Method C (yield = 71%). ^{1}H NMR (DMSO-d_6) δ 8.52 (d, 1H, J = 7.9 Hz), 8.10 (br s, 3H), 7.88 (br s, 1H), 7.56 (m, 2H), 7.45 (m, 2H), 7.39–7.25 (m, 5H), 5.65 (m, 1H), 4.71 (m, 1H), 4.52 (d, 1H, J = 16.0 Hz), 4.42 (s, 2H), 4.07 (d, 1H, J = 16.0 Hz), 3.77 (m, 1H), 3.61 (m, 1H), 3.35 (m, 1H), 3.18 (m, 1H), 3.07 (m, 2H), 1.47–1.32 (m, 9H), 0.90 (m, 3H). MS m/z 593 (MH^+).

5.4.88. 2-(*R*)-2-(2-(*R*)-2-Aminopropionylamino)-3-phenylmethoxypropionic acid *N*-(5-(4-chloro-phenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide (28). The title compound was prepared from **6** and *N*-Boc-D-alanine as described in Method C, but isolated after extraction of the ether layer with $NaHCO_3$ as the free base (yield = 43%). ^{1}H NMR ($CDCl_3$) δ 7.77 (m, 1H), 7.47–7.19 (m, 8H), 4.96 (m, 1H), 4.64 (m, 1H), 4.46 (s, 2H), 4.33 (m, 1H), 4.15 (m, 1H), 3.55–3.39 (m, 3H), 3.30 (m, 1H), 3.09 (m, 1H), 1.55–1.43 (m, 6H), 1.29 (t, 3H), 0.89 (m, 3H). MS *m*/*z* 564 (MH^+)

5.4.89. 2-(*R*)-2-(2-(*S*)-2-Aminopropionylamino)-3-phenylmethoxypropionic acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide (29). The title compound was prepared from **6** and *N*-Boc-L-alanine as described for **28** (yield = 40%). ^{1}H NMR ($CDCl_3$) δ 7.77 (m, 1H), 7.47–7.19 (m, 8H), 4.96 (m, 1H), 4.64 (m, 1H), 4.46 (s, 2H), 4.33 (m, 1H), 4.15 (m, 1H), 3.55–3.39 (m, 3H), 3.30 (m, 1H), 3.09 (m, 1H), 1.55–1.43 (m, 6H), 1.29 (t, 3H), 0.89 (m, 3H). MS *m*/*z* 564 (MH^+).

5.4.90. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-4-phenylmethoxybutyric acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (30). The title compound was prepared from *N*-Boc-*O*-benzyl-D-homoserine as described in Methods B and C (yield = 9%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.56 (m, 2H), 7.44 (m, 2H), 7.38–7.25 (m, 5H), 4.61 (m, 2H), 4.40 (m, 2H), 3.94 (d, 1H, J = 16.3 Hz), 3.33 (m, 2H), 3.03 (m, 2H), 1.58–1.32 (m, 14H), 0.95 (m, 3H). MS *m*/*z* 591 (MH^+).

5.4.91. 2-(*R*)-2-(2-Amino-2-methylpropionylamino)-4-phenylbutyric acid *N*-(5-(4-chlorophenyl)-3,3-dimethyl-1,1-dioxo-2,3-dihydroisothiazol-4-ylmethyl)-*N*-ethylamide hydrochloride (31). The title compound was prepared from *N*-Boc-D-homophenylalanine as described in Methods B and C (yield = 51%). ^{1}H NMR (DMSO-d_6 + D_2O) δ 7.50 (m, 2H), 7.39 (m, 2H), 7.32–7.10 (m, 5H), 4.49 (d, 1H, J = 16.3 Hz), 4.30 (m, 1H), 4.04 (m, 1H), 2.84 (m, 1H), 2.69 (m, 1H), 2.40 (m, 2H), 1.59 (m, 1H), 1.42–1.34 (m, 6H), 1.23–1.13 (m, 7H), 0.75 (m, 3H). MS *m*/*z* 561 (MH^+).

5.5. GH release in pituitary cells

Anterior pituitaries from male Sprague–Dawley rats were sectioned into small pieces and digested enzymatically using trypsin (Difco). Pituitary cells were dispersed by mechanical agitation, collected, pooled and then seeded into 96-well plates (50,000 cells/well). After 5 days of culture, the cells formed as monolayer (70–80% confluent). Cells were then washed with medium and incubated for 90 min at 37 °C. Then the cells were challenged to secrete GH by the addition of GH secretagogues to the medium. After 45 min at room temperature, the medium was removed, filtered and stored frozen until radioimmunoassays for rat GH were performed. EC_{50} values were calculated as an average of at least three runs and are represented as means ± standard error, unless otherwise stated. All presented compounds caused a release of GH as a statistically significant increase (at least 20%) of the basal level.

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